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Validation of a FVIII Chromogenic Nijmegen Bethesda Assay for the Detection of Inhibitors in the Presence of Emicizumab (ACE-910)

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Inhibitors

Abstract

Background: The measurement of FVIII inhibitors is important in the management of Hemophilia A patients and is commonly performed using the Classical Bethesda assay based on the one-stage clotting method. It has been reported that FVIII inhibitor measurement in Hemophilia A patients receiving Emicizumab (ACE-910) therapy show drug interference, leading to false negative inhibitor titers when using a one-stage based factor inhibitor assay. Therefore, there is a need to validate a Nijmegen Bethesda method that is not affected by the presence of Emicizumab, a bivalent antibody bridging activated FIX and FX. Objectives: To validate a FVIII Chromogenic Nijmegen Bethesda assay (C-NBA) for measurement of FVIII inhibitors in Hemophilia A patients receiving Emicizumab (ACE-910) therapy. Methods FVIII activity was measured using the Siemens FVIII Chromogenic Assay on the BCS ® XP (Siemens Healthcare Diagnostics Inc.). Dilutions of inhibitor samples were prepared in 50 mM imidazole buffer containing 4% Bovine Serum Albumin (BSA). Sheep anti-human FVIII inhibitor plasma (Affinity Biologicals) was spiked into congenital FVIII deficient plasma to obtain inhibitor validation samples at 40.0, 5.0, 1.0 and 0.25 (negative) CBU/mL. Validation samples were tested in the FVIII Chromogenic Nijmegen Bethesda assay post heat pre-treatment at 56 ± 2°C for 60 minutes. Intra- and inter-assay relative accuracy and precision, as well as dilution integrity, selectivity and sample stabilities were assessed. In addition, a second set of inhibitor validation samples at 10.0, 5.0, 1.0 and 0 CBU/mL, containing either 50 or 100 µg/mL Emicizumab, were prepared and tested. Results Intra- and inter-assay relative accuracy and precision was demonstrated for all levels of positive inhibitor samples. Intra- and inter-assay relative accuracy (RE) ranged between -22.0 to 0.4% and -20.0 to -0.2%, respectively. Intra- and inter-assay precision (CV) for the positive validator samples ranged between 8.8 to 19.6% and 11.4 to 16.1%, respectively. Dilution integrity was demonstrated by testing a high inhibitor sample at four separate pre-dilutions. Selectivity was demonstrated in six congenital FVIII deficient donor plasmas spiked to 1.0 CBU. Sample stability was demonstrated up to 3 hours post heat treatment and 2 hours post incubation, as well as following 3 additional freeze thaw cycles. Emicizumab at 50 or 100 µg/mL did not result in a change to the measured inhibitor titer when compared to a buffer control. Conclusion: The FVIII Chromogenic Nijmegen Bethesda assay (C-NBA) was successfully validated for the measurement of FVIII inhibitors in Hemophilia A patients in the presence of Emicizumab (ACE-910).